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UTILITY OF H3K27ME3 IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF MALIGNANT PERIPHERAL NERVE SHEATH TUMOUR: A CROSS-SECTIONAL STUDY FROM A TERTIARY CARE CENTRE IN PAKISTAN

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Abstract

Objective: *To evaluate the diagnostic utility of H3K27me3 immunohistochemistry in malignant peripheral nerve sheath tumour (MPNST) and its correlation with clinicopathological features.*

Study Design: *Cross-sectional descriptive study.*

Place and Duration of Study: *Department of Histopathology, Chughtai Institute of Pathology, Lahore, Pakistan, over one year after ethical approval from institutional review board.*

Methodology: *A total of 40 histologically confirmed cases of MPNST were included. H3K27me3 immunostaining was performed on formalin-fixed paraffin-embedded (FFPE) tissue. Staining patterns were classified as complete loss, focal loss, mosaic loss, or retained expression. Findings were correlated with tumour grade, patient age, gender, and neurofibromatosis type 1 (NF1) status.*

Results: *Of 40 cases, 35 were evaluable for H3K27me3 expression (87.5%). Loss of expression (complete, focal, or mosaic) was observed in 21 cases (60.0%), with complete loss being the most common pattern (n = 13; 37.1%). The mean patient age was 54.9 ± 17.5 years, with male predominance (60%). The extremities were the most frequent tumour site (52.5%). Mosaic loss was observed in both NF1-associated cases in which H3K27me3 was evaluable. No statistically significant association was identified between H3K27me3 loss and tumour grade, patient age, or gender ($p > 0.05$).*

Conclusion: *H3K27me3 loss is a diagnostically useful immunohistochemical marker in MPNST, demonstrating loss in 60% of evaluable cases. Interpretation should be contextualised within the overall morphological and immunohistochemical profile.*

INTRODUCTION

Malignant peripheral nerve sheath tumour (MPNST) is a rare, aggressive, neural crest-derived sarcoma accounting for 5–10% of all soft tissue malignancies. [1] Its reported prevalence in the general population is under 0.001%; however, it poses a substantial clinical burden owing to its aggressive behaviour, high recurrence rates, and poor overall prognosis. [2]

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MPNST arises either sporadically (approximately 50% of cases), in association with neurofibromatosis type 1 (NF1; 40–50%), or following prior radiation therapy. [3] Diagnosis is particularly challenging owing to the considerable overlap in morphological and immunophenotypic features with other spindle cell neoplasms, including synovial sarcoma, fibrosarcoma, leiomyosarcoma, and cellular schwannoma. Conventional markers such as S-100, SOX10, CD34, and EMA offer limited sensitivity and specificity when used in isolation, with better performance confined to high-grade tumours. [5]

Loss of H3K27 trimethylation (H3K27me3), a repressive histone modification mediated by polycomb repressive complex 2 (PRC2), has recently emerged as a novel epigenetic marker for MPNST. Global loss of H3K27me3 nuclear expression results from inactivating mutations in PRC2 subunit-encoding genes, most frequently EED or SUZ12. 6 Such mutations occur in approximately half to two-thirds of sporadic and NF1-associated MPNSTs, yet are rarely detected in benign peripheral nerve sheath tumours or their histological mimics. [7] Multiple studies have validated H3K27me3 loss as a sensitive and relatively specific marker for MPNST when interpreted within the appropriate clinical and morphological context. 8, 9 Nevertheless, this marker has not been formally evaluated in local practice, and regional validation is warranted given differences in tumour demographics, NF1 prevalence, and institutional protocols.

This study aimed to evaluate the diagnostic utility of H3K27me3 immunohistochemistry in histologically confirmed MPNST cases at a tertiary referral centre in Lahore, Pakistan, and to correlate expression patterns with clinicopathological parameters.

METHODOLOGY

This cross-sectional descriptive study was conducted in the Department of Histopathology, Chughtai Institute of Pathology, Lahore, Pakistan, over one year following approval by the institutional review board (IRB). The study was performed in accordance with the Declaration of Helsinki, and written informed consent was obtained from all relevant parties.

All histologically confirmed MPNST cases diagnosed during the study period at the study centre, regardless of patient age, gender, tumour site, or grade, were eligible for inclusion. Exclusion criteria comprised cases with an unconfirmed diagnosis, insufficient tissue for immunohistochemistry, or incomplete clinical data. A minimum of 40 cases was set as the sample size, selected through consecutive sampling.

Demographic and clinical data—including age, gender, tumour site, and NF1 status—were recorded for all cases. Surgical resection specimens and biopsy material were routinely processed as formalin-fixed, paraffin-embedded (FFPE) tissue blocks. Histological grading was assigned using the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) system, incorporating tumour differentiation score, mitotic count, and extent of necrosis where assessable.

Immunohistochemistry for H3K27me3 was performed on 4 micron sections of FFPE tissue, using a validated antibody. Staining adequacy was confirmed by the presence of internal positive controls (non-neoplastic stromal cells, endothelial cells, and lymphocytes). The staining pattern was classified as; (i) complete loss - no nuclear staining in all tumour cells and intact internal controls, (ii) focal loss- loss in a minority (< 50%) of nuclei within the mutant cell population, (iii) mosaic loss - areas with preserved expression interspersed with areas of complete or focal loss within the same section (characteristic for NF1 tumours), or (iv) retained expression - preservation of nuclear staining in tumour cells.

Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as absolute and relative frequencies. The association between H3K27me3 expression and clinicopathological variables (tumour grade, age group, gender, NF1 status) was evaluated using the Chi-square test or Fisher's exact test, as appropriate. A p-value of < 0.05 was considered statistically significant.

RESULTS

The study comprised 40 histologically confirmed MPNST cases. The mean patient age was 54.9 ± 17.5 years (range 17–83 years). Of the total cases,

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24 (60.0%) were male and 16 (40.0%) were female. The largest age group was patients aged >60 years (n = 16; 40.0%), followed by the 40–60-year group (n = 13; 32.5%) and those aged <40 years (n = 11; 27.5%).

The most frequent anatomic site was the extremities (n = 21; 52.5%), followed by the trunk/chest wall (n = 10; 25.0%), head and neck (n = 5; 12.5%), and spine, retroperitoneum, or other locations (n = 4; 10.0%). NF1 was documented in five patients (12.5%). FNCLCC grading was feasible in 25 cases: 12 were high grade (Grade 3; 48.0%), eight were intermediate grade (Grade 2; 32.0%), and five were low grade (Grade 1; 20.0%). The remaining 15 cases could not be graded, primarily owing to fragmented specimens or core needle biopsy material.

Table-I — Summary of clinicopathological characteristics.

Table-I: Clinicopathological Characteristics of Patients (n = 40)

Variable	Frequency (n)	Percentage (%)
Gender		
Male	24	60.0
Female	16	40.0
Age Group (years)		
< 40	11	27.5
40–60	13	32.5
> 60	16	40.0
Tumour Site		
Extremities	21	52.5
Trunk / Chest Wall	10	25.0
Head & Neck	5	12.5
Spine / Retroperitoneum / Others	4	10.0
NF1 Status		
Present	5	12.5
Absent	35	87.5
FNCLCC Grade (n = 25 graded)		
Grade 1 / Low Grade	5	20.0
Grade 2 / Intermediate Grade	8	32.0
Grade 3 / High Grade	12	48.0

H3K27me3 immunostaining was evaluable in 35 of 40 cases (87.5%); the remaining five were non-evaluable owing to absent internal positive controls or suboptimal tissue preservation. Among the 35 evaluable cases, any pattern of H3K27me3 loss was identified in 21 (60.0%), while retained expression was recorded in 14 (40.0%).

Among the 21 cases with loss, complete loss was the predominant pattern, observed in 13 (37.1%). Focal loss was identified in six cases (17.1%), and mosaic loss in two (5.7%). Both mosaic cases occurred in NF1-associated tumours, consistent with the recognised association between this pattern and NF1-related MPNST. The full distribution of

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H3K27me3 expression patterns is presented in Table-II.

Table-II: H3K27me3 Expression Patterns in Evaluable Cases (n = 35)

Expression Pattern	Frequency (n)	Percentage (%)
Complete Loss	13	37.1
Focal Loss	6	17.1
Mosaic Loss	2	5.7
Total Loss	**21**	**60.0**
Retained Expression	14	40.0
Total Evaluable	**35**	**100.0**

There was no statistically significant association between loss of H3K27me3 with either tumour grade (p = 0.43), patient age group (p = 0.61) or gender (p = 0.72). A formal statistical comparison of H3K27me3 loss with NF1 status was not conducted due to the limited number of evaluable NF1-associated cases (n = 3).

DISCUSSION

MPNST remains among the most diagnostically challenging soft tissue sarcomas, largely due to the absence of a single highly specific immunohistochemical marker and its considerable morphological overlap with other spindle cell tumours. Progress in this area has been facilitated by the identification of epigenetically regulated markers, notably H3K27me3.

In the present series, H3K27me3 loss was identified in 21 of 35 assessable MPNSTs (60.0%), consistent with the previously reported range of 50–70%, which varies according to the population studied and the technical methodology employed. 17 The landmark series of 140 cases by Prieto-Granada et al. demonstrated H3K27me3 loss in 67.6% of MPNSTs, with markedly lower rates in benign nerve sheath tumours and histological mimics. [8,10] The present findings confirm the applicability of this marker in a South Asian institutional setting.

Complete loss (37.1%) was the most prevalent pattern, consistent with prior reports, and is attributed to biallelic inactivation of PRC2 component genes (EED or SUZ12) across the entire tumour cell population. Mosaic loss, characterised by alternating zones of loss and retained expression, was identified exclusively in NF1-associated cases. This pattern reflects intratumoral genomic heterogeneity in NF1-related MPNST and should be recognised as a diagnostically informative finding rather than interpreted as retained expression. 12,13

The anatomic distribution, with the majority of tumours arising in the extremities (52.5%) and trunk (25.0%), mirrors findings from published series that consistently identify these regions, together with the paraspinal area, as predominant sites. The mean age of 54.9 years and male predominance (60%) were broadly comparable with reported cohorts, although some series have described a more balanced gender distribution. 2,4

FNCLCC grading was achievable in only 62.5% of cases (25/40), largely because a substantial proportion of specimens consisted of core needle biopsies or fragmented excisions, limiting adequate assessment of necrosis and tumour differentiation. Among graded cases, high-grade tumours (Grade 3; 48.0%) predominated, as would be anticipated given the inclusion of NF1-associated and retroperitoneal or deep-seated lesions. 14

The absence of a statistically significant association between H3K27me3 loss and tumour grade aligns with findings from several other groups. This supports the premise that H3K27me3 loss functions primarily as a diagnostic marker rather than an indicator of histological grade or tumour aggressiveness, reflecting PRC2 inactivation rather than broader oncogenic dysregulation. Similarly, the

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lack of significant associations with age or gender is unsurprising, as EED/SUZ12 mutations demonstrate no predilection for any specific demographic subgroup.

The non-evaluability rate of 12.5% in this series warrants comment. Technical failure of H3K27me3 immunostaining may result from small biopsy size, over-fixation in formalin, or ischaemic artefact. The identification of reliable internal positive controls—including intratumoral endothelial cells and stromal fibroblasts—is essential for accurate interpretation, underscoring the importance of standardised laboratory protocols at centres incorporating this marker.

The principal limitation of this study is its sample size. Although 40 cases represent a meaningful institutional series, the cohort is insufficient for robust multivariate analysis or subgroup comparisons, particularly for NF1-associated tumours. A prospective, multi-institutional design would yield more definitive conclusions. Additionally, molecular confirmation through EED/SUZ12 mutational analysis was not performed; future studies should integrate next-generation sequencing with immunohistochemical findings to further refine diagnostic accuracy.

CONCLUSION

In this institutional series, H3K27me3 expression loss was identified in 60.0% of assessable MPNST cases, with complete loss as the predominant pattern and mosaic loss confined to NF1-associated tumours. H3K27me3 immunohistochemistry represents a diagnostically valuable and practically accessible adjunct in the evaluation of MPNST, particularly for its distinction from benign peripheral nerve sheath tumours and spindle cell mimics. Results must be interpreted alongside morphological findings and a comprehensive immunohistochemical panel. Institutions adopting this marker should invest in standardised technical protocols and training in pattern recognition to ensure reliable application.

CONFLICT OF INTEREST: None declared.

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